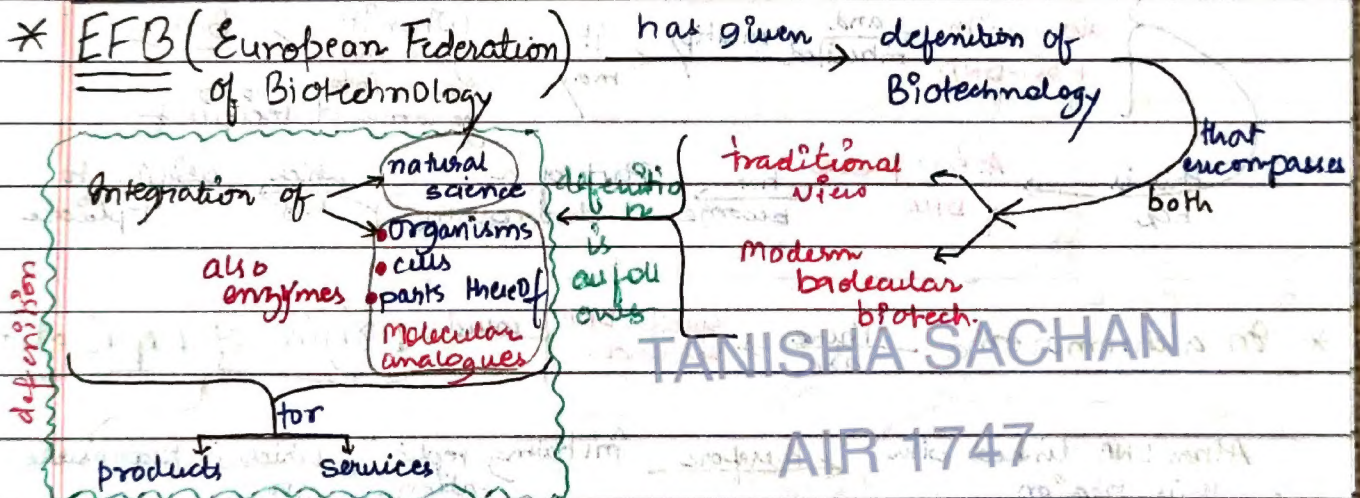
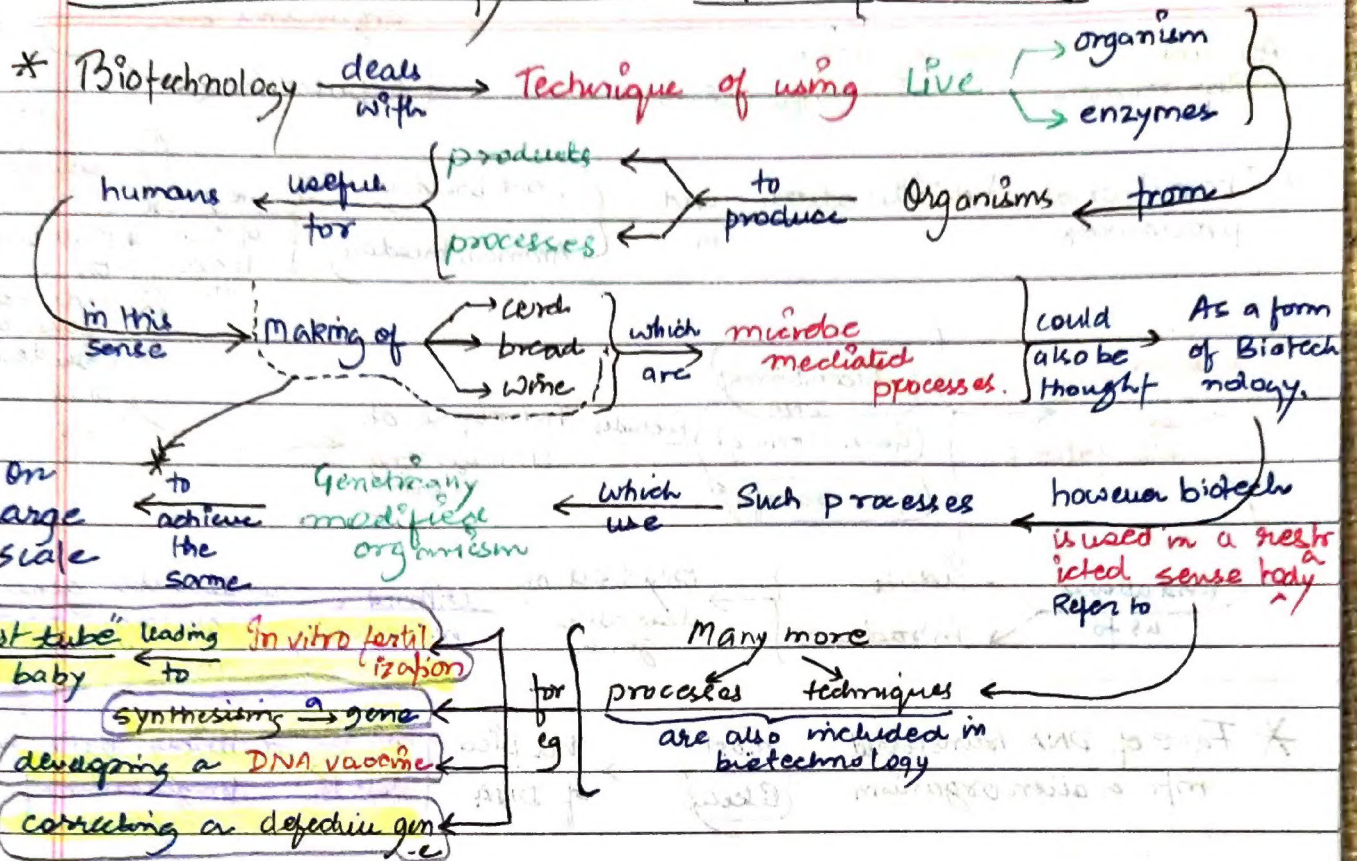


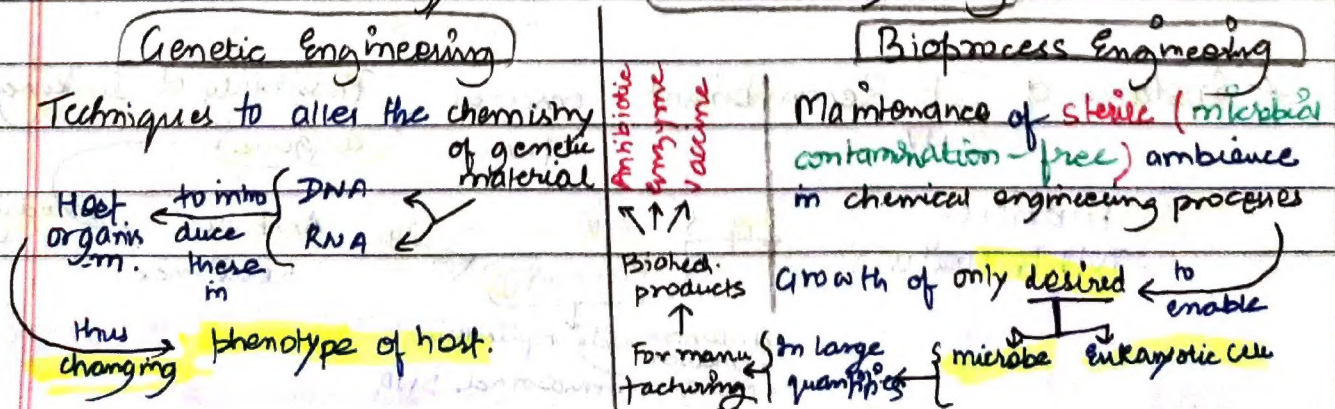
Biotechnology & its Processes

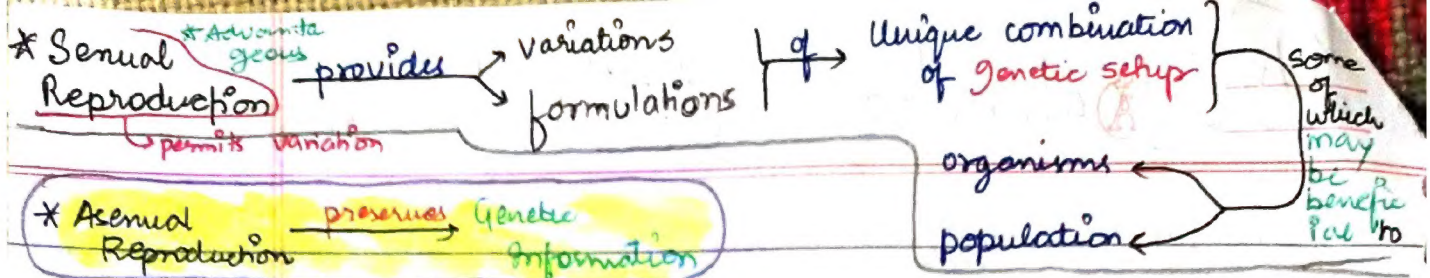


Principles Of Biotechnology

* Among many the 2 core technique enabling birth of biotechnology

- Genetic Engineering
- Bioprocess Engineering





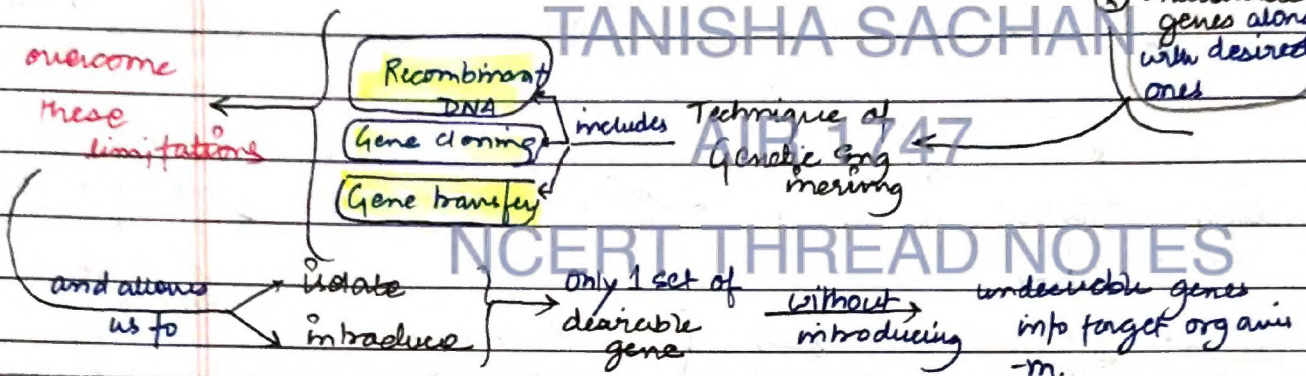
* Traditional Hybridisation used in plant breeding & Animal breeding

very often lead to

(1) Inclusion

(2) Multiplication of undesirable genes along with desired ones

overcome these limitations

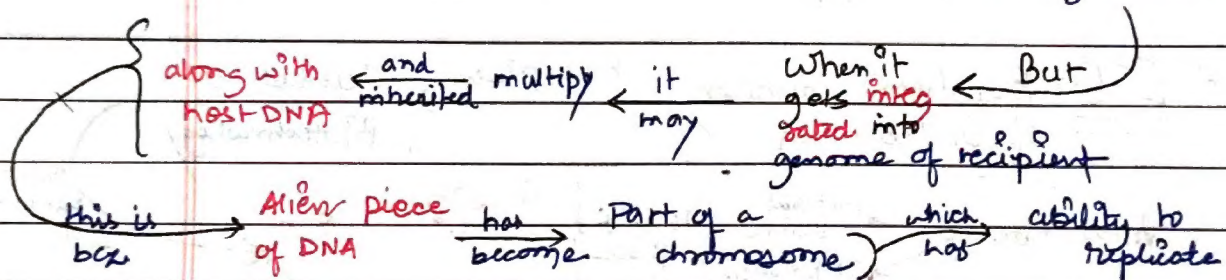


* Fate of DNA transferred into a alien organism

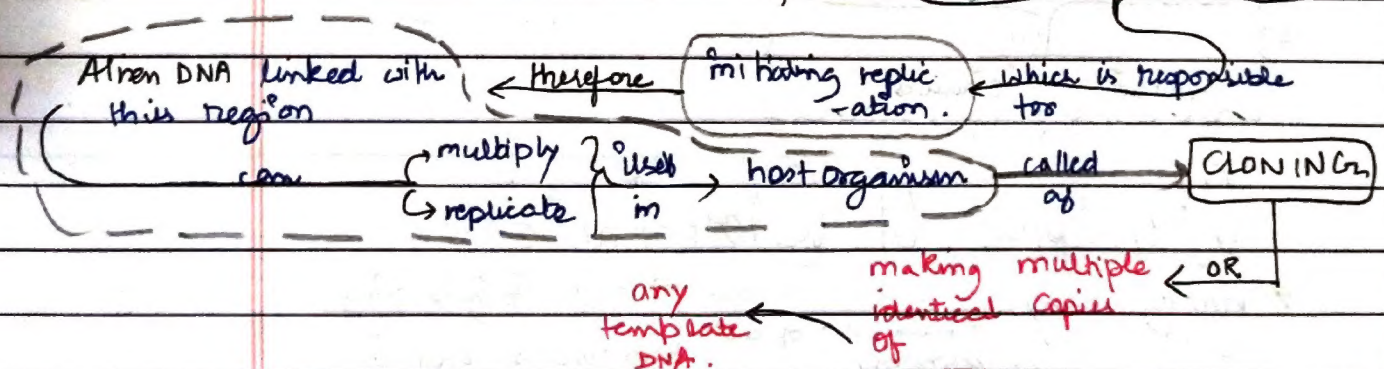
most likely

this piece of DNA

not be able to multiply itself in progeny cells of organism



* In a chromosome there are specific DNA sequence called Origin of Replication



* Instance of 1st Recombinant DNA emerged from Possibility of linking a gene

Salmonella typhimurium

of native PLASMID

With Antibiotic Resistance

encoding

- autonomously replicating
- circular
- extra-chromosomal DNA

Stanley Cohen & Herbert Boyer

accomplished this in 1972

by

isolating the antibiotic resistance gene.

by

cutting out a piece of DNA

discovery of

so called MOLECULAR SCISSORS

became possible with

at specific locations

this cutting of DNA

Plasmid which was responsible for conferring antibiotic resistance.

Restriction Enzymes.

* Cut piece of DNA

was linked to

Plasmid DNA

acts as

VECTORS

to transfer

piece of DNA attached to it

Host organism

deliver an alien piece of DNA

to Vector

hence plasmid is used as

* Linking of antibiotic Resistance gene

to plasmid vector

becomes possible with

Enzyme - DNA Ligase

New combination

this makes

joins their ends

and

cut DNA molecules

which acts on

Circular autonomously replicating DNA

created

In vitro

known as

Recombinant DNA.

multiple copies and make

Replicate using it host's DNA polymerase

Escherichia coli

when this DNA was transferred into

A bact. closely related to Salmonella.

The ability to multiply copies of antibiotic resistance gene in E. coli

was called

Cloning of antibiotic resistance gene in E. coli

* 3 basic steps in Genetically Modifying an organism →

① Identification of DNA with desirable genes

② Introduction of identified DNA into host

③ Maintenance of introduced DNA in host & transfer of it into progeny

Tools OF RECOMBINANT DNA TECHNOLOGY.

Key tools for Genetic Engineering OR Recombinant DNA tech

- restriction enzyme
- polymerase enzyme
- Ligase
- Vectors
- Host organism

Restriction Enzymes

In 1963 → 2 Enzymes responsible for restricting the growth of bacteriophage in *E. coli* were isolated.

Other, cut DNA

RESTRICTION ENDONUCLEASE

One, added -CH₃ gp to DNA

1st Restriction Endonuclease — Hind - II — whose functioning is depended on a specific DNA nucleotide sequence

Hind - II always cut DNA molecules at a particular point characterised by a specific sequence of 6 base pairs. This is known as Recognition Sequence.

Hind - II was found 5 years later was isolated & characterised.

Besides Hind - II, we know > 900 — Restriction Enzymes that have been isolated from 230 strains of bacteria which recognise different recognition sequences.

Convention For naming these enzymes

① First letter comes from Genus
② Second 2 letters comes from Species of Prok. cell from which they were isolated.

Example

EcoRI — comes from *Escherichia Coli* RY13.
Roman no. indicates order in which enzymes were isolated.
designed from — Name of strain

Restriction Enzymes

belong to a larger class of enzymes — NUCLEASES — that strain of bact. from

Exonuclease

Remove nucleotide

from.

Ends of DNA

Endonucleases

Cuts at specific positions

within DNA.

Each Restriction Endonuclease functions by "recognising" the length of DNA sequence. Will bind to the DNA & once it finds specific recog. sequence.

Cut each of the 2 strands of the DNA → Double helix → at → Specific points in → sugar phosphate backbone

* Each Restriction Endonuclease recognises → Specific palindromic nucleotide sequences in the DNA.

Base pairs that read same on 2 strands when orientation of reading is Rev't the same. In DNA sequence These are groups of letters that form same words when read backward & forward - **PALLINDROMES**

Example → 5' → GAATTC → 3'
3' ← CTTAAG ← 5'

* Restriction Enzymes cut the strand of DNA

→ A little away from the centre of the palindromic sites.

Single stranded portion at end. → these are → Overhanging stretches → called → **STICKY ENDS** (on each strand)

but b/w the same 2 bases on opposite strands → this leaves → they form H-bond with their complementary cut counterparts

*** Sticky ends facilitate the action of ligase**

* Restriction endonucleases used in → Genetic Engineering → to form → Recombinant molecules of DNA

DNA from different source / genome → which are composed of

* When cut by → Same restriction Enzyme → resultant DNA fragments

↓ have → DNA ligase ← can be joined by → Same kind of sticky ends.

Hence → unless one cuts → vector } by → Same restriction Enzyme
 Source DNA
 Recombinant vector cannot be created

Separation & Isolation of DNA fragments

① Cutting of DNA → by restriction endonuclease results in → fragments of DNA → can be separated by technique → Gel Electrophoresis

② DNA fragments → are → -vely charged molecule / DNA can be → Separated by forcing them to move towards Anode

these days used → Medium / Matrix ← through → Electric field ← under

Agarose gel
 (natural polymer extracted from seaweeds)

DNA fragments separate (resolve) accn to their size only

Smaller the fragment → Farther it moves

sieving effects ← through → provided by agarose gel

Separated DNA fragments → can be → visualised only after staining with → Ethidium Bromide

DNA fragments can be seen bright orange coloured bands

Exposure to UV radiation → followed by

In Ethidium bromide stained gel exposed to UV light

Elution

Separated bands of DNA

cut out from → Agarose gel

Gel pieces ← extracted from

* DNA fragments → purified this way are → used in constructing → Recombinant DNA

Cloning vectors → by joining them with

Cloning Vectors

Plasmids → have ability to replicate → within bacterial cells independent of control of chromosomal DNA
 Bacteriophage

bacteriophage $\xrightarrow{\text{bcz of them}}$ high copy no. per cell. $\xrightarrow{\text{have}}$ very high copy numbers

Bacterial cells $\xrightarrow{\text{within of their genome}}$

Some plasmids may have only 1 or 2 copies per cell whereas others have 15-100 copies per cell.

their no's can go even higher

* If we can \rightarrow Link alien piece of DNA with Bacteriophage Plasmid DNA \rightarrow we can Multiply the numbers equal to copy no. of plasmid Bacteriophage.

* Vectors used at present \rightarrow Are engineered in such a way

① easy linking of foreign DNA

① they help in

and ② Selection of recombinants from non recombinants

The following are features that are required to facilitate cloning into a vector:

(i) ORIGIN OF REPLICATION (ORI)

\rightarrow Is a sequence where Replication starts

* Any piece of DNA linked to this sequence $\xrightarrow{\text{can be made to replicate}}$ within host cell.

* This sequence is responsible for \rightarrow controlling copy no. of linked DNA

if one wants to recover many copies of the target DNA

\rightarrow It should be Cloned in a vector having high copy no.

(ii) SELECTABLE MARKER

In addition to \rightarrow "ori", vector requires a selectable marker.

Selectively permitting the growth of transformants

non-transformants

which helps in
① identifying
② eliminating

* TRANSFORMATION is a procedure through which a piece of DNA is introduced in Host Bacteria

* Useful Selectable Marker for E. coli \rightarrow Genes encoding resistance to antibiotics
— ampicillin
— chloramphenicol
— tetracycline
— Kanamycin

(ii) Cloning Sites :

In order to link alien DNA

vector needs to have

Very few, probably single Recognition sites.

Restriction enzyme

← for commonly used

* Presence of more than one recognition sites

within vector will

Generate several fragments

↓
which will complicate the gene cloning

* Ligation of alien DNA is carried out at a restriction site

present in one of the 2

Antibiotic Resistance genes

Imp. Example

Ligate a foreign DNA

at

BamH I site of Tetracycline resist. gene (in Vector - PBR322)

Recombinant plasmids lose the tetracycline Resistance

non-recombinants

Can be selected out from

BUT STILL

Insertion of foreign DNA

due to

by

plating the transformants

on

Tetracycline medium

* Transformants

growing on

ampicillin medium

are then transferred to

Tetracycline medium

* RECOMBINANTS

grows in Ampicillin med.

not grows in Tetracycline med.

* NON-RECOMBINANTS

grows on

Both antibiotics medium

1 antibiotic resistance gene

helps in

Selecting Transformants

other antibiotic resistance gene

gets

"inactivated" due to "insertion" of alien DNA

Selecting RECOMBINANTS

← helps in

* Selection of Recombinants due to inactivation of antibiotics

cumbersome process

bcz

it requires → **Simultaneous plating** on 2 plates having diff. antibio. res.

therefore

A **alternative selectable marker** have been developed

↓ which differentiates
recombinants from non-recombinants.

↓ on the basis of
their ability to produce colour

↓ in the presence of
A chromogenic substance/substrate

↓ In this
A recombinant DNA is inserted **within** coding sequence.

β-Galactosidase ← of enzyme

↓ This results in
Inactivation of the gene for synthesis of enzyme

↓ referred to as
Insertional inactivation

* Presence of chromogenic **gives** Blue coloured colonies if Plasmid in Bact. doesn't have insert.
Substrate

* If insert is present results in **Insertional inactivation** of β-galactosidase gene
recombinants ← these are colonies do not produce any colour

(iv) Vectors for Cloning Genes in plants & Animals

Genes can be transferred from Bacteria/Virus to plants/animals

○ How to deliver genes in **eukaryotic cells** to transform them & force them to do what bact./fungi wants.

Examples →

1) **Agrobacterium Tumefaciens** → A pathogen of several dicot plants

is able to deliver → A piece of DNA known as **T-DNA**

to transform

A normal plant cell

Produce chemicals required by pathogen

direct those tumor cells to

TUMOR

into

•) Retroviruses → In animals have ability transform normal cell to cancerous cells

* A better understanding of Art of delivering genes by pathogen has generated knowledge to transform these tools of pathogen into useful vectors for delivering genes of interest to humans

* Tumor inducing (Ti plasmid) of Agrobacterium Tumefaciens has now been modified into cloning vector which is no more pathogenic to plants but still be able to use mechanisms to deliver the gene of interest into variety of plants

* Retroviruses → now been disarmed are now used to deliver desirable gene

* Once a gene / DNA fragment → has been ligated into animal cell then transferred to suitable vector which can be multiplied in Bact. host, Animal host, or plant host

COMPETENT HOST for Transformation with Recombinant DNA

(DNA) → Hydrophobic molecules it cannot pass through cell membrane.

Bact. cells must be made competent to take up DNA in order to force bacteria to take up DNA

* Treating Bacterial cell with specific concentration of divalent cation

DNA enters bacterium through pores in cell wall which is effective with Ca^{2+} (e.g. Ca^{2+}) by incubating them (cells) with Recomb. DNA. Recombinant DNA can then be forced into such cell.

On ice $\xrightarrow{\text{followed by}}$ placing them briefly at 42°C (heat shock) $\xrightarrow{\text{then putting back on ice}}$

Bacteria to take up Recombinant DNA \leftarrow this enables

* MICROINJECTION \rightarrow Recombinant DNA directly injected into nucleus of animal cell

Substrate for plasmids

* BIOLISTICS / GENE GUN \rightarrow Cells are bombarded with DNA coated with Gold OR Tungsten \leftarrow high velocity micro-particles

* Using DISARMED PATHOGEN VECTORS \rightarrow When these are allowed to infect cells, Recombinant DNA into host \leftarrow they transfer

PROCESS OF RECOMBINANT DNA TECHNOLOGY

① Steps of Recombinant DNA technology : in sequence

- 1) Isolation of DNA
- 2) Fragmentation of DNA by restriction endonuclease
- 3) Isolation of desired DNA fragment
- 4) Ligation of DNA fragment into vector
- 5) Transferring the recombinant DNA into host
- 6) Culturing the host cells in a medium at large scale
- 7) Extraction of desired product

(1) Isolation Of Genetic Material (DNA) :

Nucleic acid \rightarrow Genetic material of all \rightarrow without exception

* In majority of organism \rightarrow DNA

* In order to cut DNA by restriction enzymes $\xrightarrow{\text{it should be in}}$ Pure form [free from other macro molecules]

we have to break open cell \rightarrow DNA \leftarrow DNA is enclosed within membrane \rightarrow along with macromolecules \rightarrow RNA, protein, polysaccharide, lipid

this breaking of cell memb. achieved by treating \rightarrow Bacterial cell, Plant tissue, Animal tissue \rightarrow with enzymes as \rightarrow lysozyme - Bacteria, cellulase - plant cells, chitinase - fungus

* Genes located on \rightarrow Long molecules of intertwined DNA \rightarrow Proteins (as histone)

* RNA removed by \rightarrow Ribonuclease treatment

* Proteins removed by \rightarrow Protease treatment

* Other molecules can be removed by \rightarrow appropriate treatments (Lipases)

* Purified DNA \rightarrow precipitates out after addition of chilled ethanol seen as collection of fine threads in suspension
Separated by SPOOLING

(2) Cutting of DNA at specific Locations:

Restriction Enzyme (R.E) digestion are performed by incubating purified DNA molecules with Restriction enzymes

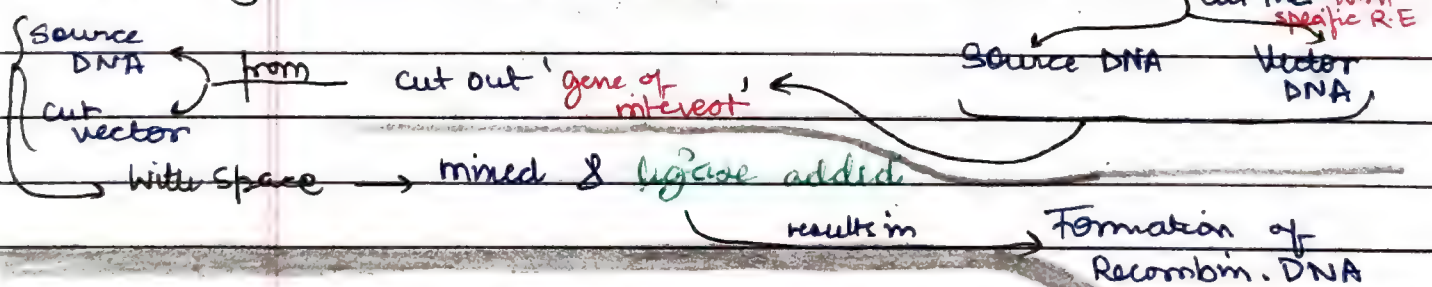
optimal condition \leftarrow at (as that specific enzyme)

* Agarose Gel Electrophoresis employed to check progression of restriction enzyme digestion

* DNA \rightarrow being negatively charged \rightarrow move toward anode

~~this process is repeated with Vector DNA also~~

* Joining of DNA involves several process



(3) Amplification of Gene of Interest using PCR:

PCR - Polymerase Chain Reaction

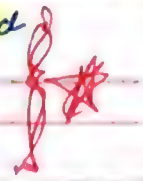
* In this rxn \rightarrow multiple copies of Gene/DNA synthesized \rightarrow in vitro

① Enzyme - DNA polymerase

② 2 sets of primers

using \rightarrow small chem. synthesized oligonucleotides Complementary to the regions of DNA

Enzyme $\xrightarrow{\text{extends}}$ primers $\xrightarrow{\text{using}}$ nucleotides provided in reaction.
Genomic DNA as template



If process of Replication of DNA $\xrightarrow{\text{repeated}}$ many times \rightarrow segment of DNA $\xrightarrow{\text{amplified to } \infty}$ Billion times (1 billion copies)

Bacteria - *Thermus Aquaticus*

isolated from

Thermostable DNA polymerase

by use of

Such repeated amplification is achieved

remains active during high temp. induced denaturation

of \rightarrow ds DNA

* Amplified fragment of desired

vector $\xleftarrow{\text{with}}$ ligase \rightarrow further cloning

(4) Insertion Of Recombinant DNA into Host Cell / Organism

Several methods of $\xrightarrow{\text{introducing}}$ ligated DNA \rightarrow into \rightarrow recipient cells \rightarrow these cells after making \rightarrow "competant"

* if Recombinant DNA bearing gene

\rightarrow for

Resistance

(present in its surrounding)

recieve & take up

to

to an

antibiotic (eg. ampicillin)

transformed to

E. coli

Transformed cells

$\xleftarrow{\text{if we spread}}$

ampicillin-resistant cells.

become host transformed cells

on

Agar plates

containing

ampicillin

\rightarrow only transformants will grow

Select a Transformed cell

$\xleftarrow{\text{one is able to}}$

due to ampicillin resistance gene

untransformed recipients will die.

in presence of

ampicillin

• Ampicillin Resistance gene

$\xrightarrow{\text{here called}}$

Selectable Marker.

(5) Obtaining the Foreign Gene Product :

When you insert a piece of **Alien DNA** into cloning vector then transfer to ^{Bact.} Plant or ^{Animal cell}

Produce a **desirable protein** ← ultimate aim is → In almost all **Recombinant technologies** alien DNA gets multiplied

Hence there is **Need for recomb. DNA** to be expressed → **Foreign Gene** gets expressed.

in **host cells** → **Expression of Foreign Genes** → **under appropriate condition**
involve understanding many technical details

After having **cloned the gene of interest** → having → **optimised condition** to induce **Expression of target protein** → one has to consider producing it

If protein encoding gene expressed in **Heterologous host** → **large scale** → on **Cells** harbouring **Cloned gene of interest**
called **Recombinant protein**

Cultures may be used for → **Small scale in laboratory** → may be grown on a

extracting → desired protein → then → **Purifying** by using → **Different separation techniques**

Cells can also be multiplied in a **Continuous culture system** wherein **used medium**

fresh medium ← while one side ← from **drained out** → other → to maintain the cells in their **physiologically most active log/exponential phase**
is added from

Larger Biomass → produces this type of culturing method

leading to → **higher yields of desired protein**

small volumes cultures ~~yield~~ → appreciable quantities of products
 • To produce Large quantities → development of Bioreactors
 processed ← can be culture ← of Large volumes ← where
 (100-1000 Litres)

Thus → Bioreactors are vessels in which Raw materials are Biologically converted
 using Specific Products (individual enzymes) etc. into
 microbial cells
 plant cells
 animal cells
 human cells

TANISHA SACHAN

* A Bioreactors provides optimal conditions for achieving desired product
 Temp.
 PH
 O₂
 Salts
 Substrate
 Vitamins
 optimum Growth Conditions
 by providing

★ MOST COMMONLY USED BIOREACTORS — STIRRING TYPE

1) Stirred tank Reactor → cylindrical curved base } to facilitate mixing of reactor contents

★ Stirrer facilitates • mixing

• Oxygen availability throughout bioreactor

2) Bioreactor has
 agitator system
 O₂ delivery system
 Foam control system
 Temp. control system
 pH control system
 Sampling ports

periodically ← can be withdrawn small volumes of culture → so that

Downstream Processing

After completion of Biosynthetic stage → Product
Ready for marketing ← before it is series of processes ← subjected through
as → product.

* Process includes separation and purification collectively referred to as Downstream processing

Product has to be formulated with suitable preservatives

Such formulations has to undergo clinical trials as in case of Drugs

Requirement - Strict Quality Control testing for each product required

Downstreaming processing and Quality control Testing vary from product to product

TANISHA SACHAN

AIR 1747

NCERT THREAD NOTES



Date _____

Restriction Endonuclease by

* Genetic Engineering is possible bcz - we can cut DNA at \rightarrow specific sites

* **KEY FACTOR** which make \rightarrow plasmid vector in genetic engineering

\rightarrow its ability to carry a foreign gene.

* sticky ends \rightarrow unpaired base pairs

* See diagram on Pg no. 196 [EcoRI cuts that GAATTC]
Pg no. 197

* Remember the steps of Recombinant DNA technology in order.

* Transposons \rightarrow Jumping genes

* Taq polymerase \rightarrow Remains active during high temp induced denaturation of dsDNA
 \rightarrow Requires primer for carrying out process of polymerisation
 \rightarrow Synthesises RNA region b/w primers
~~using dNTPs & Mg^{2+}~~ Wrong statement

* Denaturation - 94°C

Annealing - 56°C

Extension - 72°C

* Kary Mullis - PCR

* Paul Berg - Father of Genetic Engineering

* Arber, Natham, Smith
Nobel prize for discovery of Restriction Endonuclease

* Vectors \rightarrow Plasmid
 \downarrow
Phagemid \rightarrow Cosmid

* Agarose - Polymer.

* PBR322 - Artificial Vector.
 \rightarrow number that distinguishes it from other plasmid.

* Vectors Transfer Methods \rightarrow Microinjection
 \rightarrow Biolistics
 \rightarrow Gene gun

* Features of plasmids \rightarrow Transferable
 \rightarrow Independent replication
 \rightarrow Circular str.

* Total no. of restriction sites in pBR322 - 8

* Have recognition sequence of 6 base pairs \rightarrow EcoRI
 \rightarrow Hind II

* 20th century offshoot of modern biology is - Biotechnology



Boyer performed in 1969, studies on a couple of Restriction Enzyme, of E. coli.

* Palindromic sequence - when read on 2 strands in same polarity.

* Artificial recombinant DNA was constructed for 1st time using

A gene encoding antibiotic resistance.

Extrachromosomal DNA of *Salmonella typhimurium*

* tpA → clot busta / used in myocardial infarction
Interferon- α → used in treatment of Hepatitis B

* Enzymes - smaller
DNA - bigger

(i.e. Enzymes are proteins synthesised from DNA)

Q. What would be molar conc. of human DNA in human cell?

$$\begin{aligned}\text{Molar conc} &= N_A \times \text{Total no. of chromosome} \\ &= 6.023 \times 10^{23} \times 46 \\ &= 2.77 \times 10^{23} \text{ moles}\end{aligned}$$

* Eukaryotic cells - don't have ~~Restriction Endonuclease~~

* Phase of meiosis where Recombinant DNA formed - Pachytene

* Reporter Enzyme - selectable marker